

*1. There are two images in the notes that seem to contradict but I'm thinking I must be looking at it wrong. On page 85, the top slide looks like the alpha unit is exposed on the "+" end. However, on page 100 the top slide seems to display the alpha unit on the "-" end which is growing?***Note, this refers to 2012 slide 90, and has been corrected****

Good catch! It appears that there is an error in this image from text. Pg 100 slide is correct, while the alpha and beta subunits of the dimer are mis-labeled and reversed on pg 85. Alpha and beta tubulin form stable dimers that rarely dissociate. GTP on α -tubulin is buried in the dimer. GTP on the β -tubulin is exposed on the dimer and exchanges with GTP in solution. When the dimer is incorporated into the growing MT, the GTP on the β -tubulin is buried and hydrolysis to GDP is prompted. MTs are polar because the dimers are all oriented the same way; β -tubulin towards the plus end, α -tubulin towards the minus end. The plus end grows faster than the – end in solution.

2. On the second lecture you gave us in the slide talking about microtubule assembly and disassembly it mentions something about C_c^+ and C_c^- and free GTP- alpha beta tubulin dimers (its the 4th bullet point). What were you referring to there?

C_c^+ and C_c^- refer to the critical concentration of tubulin dimers for the + and – ends of the MT. The propensity of tubulin subunits to assemble into MTs is dependent upon their affinity for MT ends. In order to achieve microtubule polymerization the value of this affinity (called the critical concentration, CC) has to be less than the total tubulin concentration. Because of this parameter, pure tubulin will not generally polymerize at protein concentrations below 10 $\mu\text{g/ml}$. At concentrations above the CC, tubulin will polymerize until the free subunit concentration is equal to the CC value. Free GTP alpha beta tubulin dimers refers to the requirement for GTP-bound dimer to facilitate the addition of the dimer to the growing end of the MT filament.

Also, on that same lecture I was wondering how the microtubules can grow on the negative end even though its anchored to the MTOC?

Under physiological conditions within cells, most Mts are anchored/capped on their – end within the context of an MTOC, preventing growth at the negative end. Growth can occur on negative ends (albeit slowly) if the MTs have broken off an MTOC or if one is performing the reaction in a test tube with purified tubulin dimers.

3. I am slightly confused about actin assembly/disassembly. When it is assembled, what specific G-actins form the nucleus and do those actins remain as a nuclear core or does the nucleus refer simply to the middle of the F-actin chain? An actin nucleus can refer to an existing filament (either newly polymerized or recently severed) or a few G-actin monomers that have spontaneously polymerized together (an inefficient process due to quick dissociation rates of monomers). Since monomers can be added to the + or – end, the original nucleus can be tracked to the middle of the filament or one end of the filament if growth occurs only on one end.

Also, in terms of disassembly/treadmilling, my understanding is the ADF attaches to G-actin on the pointed end, which stimulates cofilin to detach the monomer, which is then dephosphorylated and directed to the barbed end by profilin - is this correct? Very close. ADF/cofilin/Thymosin are all monomer-binding proteins that maintain the pool of unpolymerized actin in cells and regulate the nucleotide-bound state of the actin monomer. They also bind monomers in the context of a filament. These three proteins inhibit ATP and ADP dissociation from the G-actin monomer. Profilin competes with these three proteins and promotes nucleotide dissociation and binding. In a pure solution of actin, ADF/cofilin increases the rate of depolymerization of F-actin from the pointed

end. ADF accelerates treadmilling- where the filament length remains the same as monomers add to the + end and dissociate from the – end- (increasing barbed end growth) by increasing the concentration of ATP-actin. ADP-actin. Profilin binds actin monomers. Profilin-bound actin complexes are directed into interactions with actin filament nucleation proteins such as formins or wasp that efficiently drive F-actin polymerization.

Furthermore, I understand the barbed end has Cap Z attached to it - does this remove and re-attach with each G-actin monomer that is attached to the growing + end? Capping proteins bind to either the barbed (+) or pointed (-) end of actin filaments, where they block subunit addition and polymerization. Therefore they can have a role in controlling filament length. CapZ capping protein binds to the plus end of the filament, inhibiting polymerization. If actin monomers continue to dissociate from the minus end, the actin filament shrinks. Cap Z is not a leaky cap, and when it is bound to the plus end, there is no growth at the plus end.

Lastly, in your clinical example, you said that the patient's gout could be treated with colchicine - I am confused about how/why this is.

Actually, no one really knows how or why colchicine works for treating gout or why it is not more toxic than it is.

4. *I was just wondering what type of event exactly counts as a "catastrophe" in which a microtubule's end would "explode"?* Catastrophe occurs once a minute under physiological conditions in cells and is a rapid depolymerization of the microtubule end at a rate of about 1000 dimers per second, shrinking the MT about 0.5um per second. Catastrophe is terminated by the rescue phase. The catastrophes liberate GDP tubulin subunits that will exchange their GDP for GTP to regenerate the GTP-tubulin pool at a concentration above the critical concentration to support elongation of the surviving MTs. Catastrophe can be driven by a

loss of a GTP-cap in one current theory, or by binding of tubulin dimers by proteins such as stathmin.

- on page 86 when talking about actin filaments for the (+) and (-) end, which is the exposed atp binding site? it is mentioned after (-) but I wasnt sure if it applies to the positive end as well. On actin filaments, the ATP-binding cleft on the monomer points towards the minus end.

5. for the Mg^{+} needed for actin assembly, is it just a cofactor?? Mg binds, along with ATP to the cleft in the actin monomer. ATP and Mg bound G-actin monomers are incorporated in the growing ends of actin filaments.

6. For microtubule Motors page 103, what is th Cc^{+} and Cc^{-} that the treadmilling is between? See Question 2

7. on page 100, for the pictures on the structure slide, shouldnt the addition be to the (+) end? and not the (-) end that is shown?

See answer to question #1. **Note: refers to 2012 slide on pg 106 which was updated**